



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

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OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

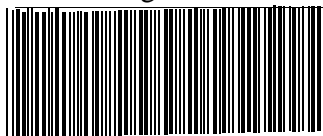
Ms. **Marian** K. Stanley  
Manager: Maleic Anhydride Panel  
Chemical Manufacturers Association  
1300 **Wilson Boulevard**  
Arlington, VA 22209

Dear Ms. Stanley:

EPA has reviewed the alternative testing proposal for **maleic** anhydride (MA) entitled: "Developing an Inhalation Testing Program for Maleic Anhydride," dated November 8, 1996, and submitted by CMA on behalf of the Maleic Anhydride Panel.

This proposal was prepared in response to EPA's invitation for proposals for pharmacokinetics (**PK**) studies for the hazardous air pollutants (**HAPs**) listed in the proposed test rule for **HAPs** (61 FR 33 178; June 26, 1996). As discussed in the proposed rule, the PK studies would be used to inform the Agency about route-to-route extrapolation of toxicity data from routes other than inhalation when it is **scientifically** defensible in order to empirically derive the inhalation risk. The PK proposals could form the basis for negotiation of enforceable consent agreements (**ECAs**) that would provide for testing in lieu of some or all of the tests proposed in the **HAPs** rule.

The following provides a background to EPA's method of evaluating the proposed PK strategies. As you recall, in the preamble to the proposed test rule, EPA indicated that, when reviewing PK proposals, it would use the Gerrity and Henry (1990) decision tree as an element in evaluating the proposed **PK** studies. The Agency also indicated that it would use mechanistic data in determining the appropriateness of route-to-route extrapolation of the existing data base as an alternative to conducting some or all of the testing required under the proposed **HAPs** test rule. Pharmacokinetics and mechanistic data may be used to inform the Agency about **route-to-route** extrapolation when EPA determines that extrapolation from existing studies may provide sufficient data to substitute for required testing under the proposed rule. Pharmacokinetics and mechanistic data may not be used alone to substitute for proposed required testing when studies by a route other than inhalation do not exist or are deemed by EPA to be inadequate. In such cases, however, pharmacokinetics and mechanistic data may be used to support a decision that required testing could be conducted using routes other than inhalation.



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EPA has concluded that this proposed strategy offers sufficient technical merit to warrant further consideration. The Agency invites the Maleic Anhydride Panel to consider EPA's preliminary technical analysis of the proposal, a copy of which is **enclosed** in this letter. Please note that this analysis, including all discussions concerning data adequacy and test procedures/methods pertains only to the adequacy of the PK proposal for its intended purpose and not to the statutory basis for issuing the **HAPs** rule under section 4 of the Toxic Substances Control Act (TSCA).

**If**, after the Panel has had the opportunity to review this analysis, you have a continued interest in pursuing the ECA process as an activity distinct **from** the test rule process, please respond to me in writing by July 31, 1997. Depending on the Panel's response, EPA will determine whether or not to proceed with the ECA process. (The procedures for ECA negotiations are described at 40 CFR 790.22(b).) Under this process, EPA would then publish a notice in the Federal Register soliciting interested parties to participate in or monitor negotiations for an ECA on maleic anhydride. The notice would also announce a date for a public meeting to negotiate the ECA. At these negotiations EPA may raise issues, based on the Agency's **further** review of the proposed strategy, that differ from those contained in the preliminary technical analysis. EPA notes that, as a result of unexpected complexities arising in the review of the PK proposals and contrary to the statement in the preamble to the proposed **HAPs** test rule, the Agency has not been able to conclude **ECAs** within 12 months of the date of the **HAPs** proposal.

The document submitted by the Maleic Anhydride Panel went beyond PK by **including** an alternate testing strategy to respond to the testing identified in the proposed **HAPs** test rule. EPA's evaluation of this proposal identifies changes or additions that provide for testing of maleic anhydride as an alternative to the testing contained in the proposed **HAPs** test rule, **If** this testing is incorporated into an ECA that is successfully concluded between EPA and the Panel, and if the data resulting from testing under the ECA are acceptable to the Agency, such testing will provide an alternative to some or all of the testing proposed for this substance in the **HAPs** test rule. If testing under the ECA does not fulfill the Agency's needs, EPA reserves the right to meet these needs through rulemaking.

EPA notes that the Maleic Anhydride Panel makes certain assumptions regarding the interpretation and use of the available toxicological database for maleic anhydride. The testing requirements for maleic anhydride in the proposed **HAPs** test rule were **identified** by EPA for the purpose of providing a database to permit the assessment of residual risk following the implementation of the maximum achievable control technology (**MACT**) standards required by the Clean Air Act. EPA must apply rigorous standards to determine the adequacy of studies to be used for route-to-route extrapolation. Although, as stated earlier in this letter, EPA considers its current analysis **of the** maleic anhydride studies to be preliminary, the Agency will be prepared to discuss all issues in detail with the Maleic Anhydride Panel if the Agency decides to proceed with the ECA process.

It is important that member companies of the Maleic Anhydride Panel recognize the importance of responding to the request for comments on the proposed **HAPs** rule. The submission of a **PK** proposal to develop an ECA to conduct testing alternative to that contained in the **HAPs** test rule is no guarantee that EPA and the Panel will, in fact, conclude such an agreement. Therefore, I urge the companies to submit comments on the **HAPs** proposed rule as an activity separate from the **ECA process**. Please submit three copies of your written comments on the proposed **HAPs** test rule, identified by document control number (OPPTS-42 187A; FRL-4869-1) to: U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Document Control Office (7407), Rm. G-099, 401 M St., SW, Washington, DC 20460.

In sum, EPA would like to thank the Maleic **Anhydride** Panel for your creative and thoughtful initial proposal. If you have any technical questions about EPA's comments on your proposal, please contact Annie Jarabek at (919) 541-4847 (voice), (919) 541-1818 (fax), or [jarabek.annie@epamail.epa.gov](mailto:jarabek.annie@epamail.epa.gov) (e-mail). For questions about the ECA process, please contact Richard Leukroth at (202) 260-0321 (voice), (202) 260-8850 (fax), or [leukroth.rich@epamail.epa.gov](mailto:leukroth.rich@epamail.epa.gov) (email).

Sincerely,



Charles M. Auer  
Director  
Chemical Control Division

Enclosure



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**Preliminary EPA Technical Analysis  
of Proposed Industry  
Pharmacokinetics (PK) Strategy for Maleic Anhydride (MA)**

July, 1997

**Chemical Name: Maleic Anhydride**

**CAS No.: 108-31-6**

**Molecular Weight: 98.06**

**Vapor Pressure: 0.1 torr at 25 °C**

**Chemical Formula: C<sub>4</sub>H<sub>2</sub>O<sub>3</sub>**

**PK Proposal Submitted by:**

The Chemical Manufacturers Association's Maleic Anhydride Panel, dated November 8, 1996, and entitled "Developing an Inhalation Testing Program for Maleic Anhydride".

**Contains No CBI**

## **Preliminary EPA Technical Analysis of Proposed Industry Pharmacokinetics (PK) Strategy for Maleic Anhydride**

### **(1) Introduction**

EPA is providing the following preliminary technical analysis and suggestions in response to a proposal by the Maleic **Anhydride** Panel (MA **Panel**) for conducting pharmacokinetics (**PK**) studies and additional toxicity testing for Maleic Anhydride (MA). This proposal was prepared **in** response to EPA's invitation for proposals for pharmacokinetics (**PK**) studies for the hazardous air pollutants (**HAPs**) listed in the proposed test rule for **HAPs** (61 FR 33 178; June 26, 1996). As discussed in the proposed rule, the PK studies would be used to **inform** the Agency about route-to-route extrapolation of toxicity data from routes other than inhalation when it is scientifically defensible in order to empirically derive the inhalation risk. **The** PK proposals could form the basis for negotiation of enforceable consent agreements (**ECAs**) that would provide for testing in lieu of some **or all** of the tests proposed in the **HAPs** rule. (The procedures for ECA negotiations are described at 40CFR **790.22(b)**). Accordingly, this analysis, including all discussions concerning data adequacy and test procedures/methods **pertains** only to the adequacy of the PK proposal for its intended purpose and not to the statutory basis for issuing the **HAPs** rule under section 4 **of the** Toxic Substances Control Act (TSCA).

Pharmacokinetics and mechanistic data may be used to inform the Agency about route-to-route extrapolation when EPA determines that **extrapolation from existing studies may** provide **sufficient** data to substitute **for required testing** under the proposed rule. Pharmacokinetics and mechanistic data alone **may not** be used to substitute for proposed required testing where studies by a route other than inhalation do not exist or are deemed by EPA to be inadequate. In such cases, however, pharmacokinetics and mechanistic data may be used to support a decision that required testing could be conducted using routes other than inhalation.

EPA acknowledges that if an ECA is successfully concluded between the Agency and the MA Panel that provides for PK studies and other testing and **if the** data resulting from testing under the ECA are acceptable to the Agency, such testing will provide an alternative to some or all of **the** testing proposed for this substance in the **HAPs** test rule. If testing under the ECA does not fulfill the Agency's needs, EPA reserves the right to meet these needs through **rulemaking**.

## (2) Toxicokinetic Properties

MA closely resembles phthalic anhydride in its potential for skin, eye, and upper respiratory tract irritation, but it is more potent as an ocular irritant. It is a known sensitizer of the respiratory tract, causing an asthma-like syndrome.. The potency of MA for induction of asthmatic response is thought to be 4-fold greater than that for phthalic anhydride. The Threshold Limit Value-Time Weighted Average (TLV-TWA) is 0.25 ppm (1  $\text{mg}/\text{m}^3$ ) (ACGIH, 1992).

Hydrolysis of MA to maleic acid (*cis-butenedioic* acid) in water is rapid; the half-life is calculated to be 36 minutes at 25.1 °C. MA is expected to rapidly hydrolyze at body temperature in all compartments within the body, including mucous layers, extracellular fluid compartments, the blood stream, tissues, and cells. It is likely that both the reactivity of MA and, the reactivity and acidity of maleic acid contribute to the irritant properties of this compound in the respiratory tract. Inhaled MA deposited from the air stream reacts with the aqueous environment of respiratory tract tissue and produces an irritant effect at low inhaled concentrations. At higher concentrations, some unreacted MA may break through the respiratory tract barrier and pass into the blood stream, but this is expected to be rapidly hydrolyzed to maleic acid. The respiratory tract deposition efficiency and high reactivity of parent MA support designation of MA as a Category 1 gas (U.S. EPA, 1994), for dosimetric adjustment of observed respiratory tract effects. Subsequent systemic distribution of maleic acid, generated by the hydrolysis of MA, raises concern for remote effects caused by maleic acid.

Oral MA exposures are reported to cause liver and kidney effects. The kidney is also the target of oral maleic acid exposures. During feeding, MA in feed becomes mixed with saliva and pancreatic secretions in the stomach. In this period, most of the MA in the feed is hydrolyzed to maleic acid. In contrast to feeding studies, there is a suggestion of direct contact site effects of MA in the stomach when delivered by gavage as a suspension in corn oil.

## (3) Proposed Maleic Anhydride Panel PK Strategy

This section describes the key aspects of the proposed ECA PK strategy entitled: "Developing an Inhalation Testing Program for Maleic Anhydride" submitted by the MA Panel.

The MA Panel proposed to perform a battery of pharmacokinetics studies for different routes of administration to demonstrate that remote tissues do not achieve a toxicologically-significant maleic acid exposure from inhaled MA. These studies are aimed at acquiring information necessary to establish that the critical toxicity after MA inhalation is limited to initial contact site in the respiratory tract, with subsequent hydrolysis and insignificant delivery of maleic acid to the blood; so that, according to the MA Panel, toxicity tests for effects of MA on systemic target organ systems are not warranted.

The MA Panel proposes to determine the rate of hydrolysis of MA at 37 °C in blood, nasal mucus, and Tris or phosphate buffer at pH 7.4. Intravenous (i.v.) dosing will be used to establish classical PK parameters such as volume of distribution, clearance, and half-life for maleic acid. Oral dosing for both MA and maleic acid at a dose equivalent to the NOAEL in the, gavage reproductive study (20 mg/kg/day) will be utilized to assess bioavailability of maleic acid based on a comparison of the area under the curve for blood (AUCB) for the i.v. versus oral dosing. The MA Panel proposes that a comparison of the AUCB for MA versus maleic acid dosing will determine the loss of MA due to reactions with other constituents in the G.I. tract versus its direct hydrolysis to maleic acid. Uptake rate in the respiratory tract would be based on the use of a measurement of the blood level of maleic acid in rats after a single concentration 6-hr inhalation exposure at a level associated with minimal structural changes in the respiratory tract (e.g., 1.1 or 3.3 mg/m<sup>3</sup>) and application of a simple zero-order in, first-order out PK model utilizing the i.v. parameters. This uptake rate would be refined by an isolated upper respiratory tract (URT) deposition study at the same concentration to determine URT extraction.

The MA Panel asserts that if the inhalation exposure does not produce maleic acid blood levels comparable to those estimated for the systemic effects, it will confirm that studies of these remote tissues are unwarranted. In addition, the MA Panel proposes that sufficient oral data exist to establish NOAEL and LOAEL levels for developmental and reproductive effects. No data on neurotoxicity or immunotoxicity are available for this comparison between routes.

To address the proposed HAPS-Test Rule data need for a 2-year carcinogenicity bioassay, the MA Panel proposes to conduct "an enhanced" 90-day subchronic study in conjunction with mutagenicity tests (*S. typhimurium*) utilizing a system to ensure gas-phase delivery of MA. The MA Panel indicates that this subchronic study will identify the NOAEL for nasal irritation, assess the reversibility of nasal lesions, and evaluate alveolar macrophage function. If the absence of mutagenicity is demonstrated, then the MA Panel proposes to drop the 2-year chronic inhalation toxicity test, since according to the MA Panel, any potential tumors would have to result from cytotoxicity and subsequent cellular proliferation as precursor events. The MA Panel asserts that cytotoxicity will be adequately characterized by the subchronic NOAEL.

Acute inhalation testing was not proposed on the basis that the potential for portalsentry effects had already been demonstrated and in consideration of animal suffering.

Table 1 compares the testing provisions described in the proposed HAPs test rule with the PK proposal submitted by the Chemical Manufacturers Association's Maleic Anhydride (MA) Panel. This table also summarizes EPA's preliminary response to the Panel's PK proposal. Detailed discussion of EPA's preliminary technical analysis are presented in section 4 of this preliminary technical analysis.

**TABLE 1. Summary Comparing Proposed Testing Provisions for MA**

Testing	Acute	Subchron	Neuro (A & SC)	Develop	Immuno Screen	Cancer/ Genetox
Proposed HAPs Rule	X	X	X	1 species	X	X
MA Panel PK Proposal	- <sup>a</sup>	X <sup>b</sup>	- <sup>c</sup>	- <sup>d</sup>	- <sup>e</sup>	- <sup>f</sup>
Preliminary EPA Response to PK Proposal	X <sup>1</sup>	X <sup>2</sup>	P(R) <sup>3</sup>	P(R) <sup>4</sup>	- <sup>5</sup>	P <sup>7</sup>

X Testing Requirement in the proposed HAPs Test Rule  
P Provisional determination  
R Route-to-route extrapolation

**Acute toxicity testing:**

-<sup>a</sup> No Alarie respiratory sensory irritation test, BAL, or acute toxicity testing proposed since MA is already established as an irritant and sensitizer. Macrophage function assay is proposed as part of 90-day study.

X<sup>1</sup> EPA maintains that respiratory tract, liver and kidney histopathology; BAL, and the macrophage function assay are needed as called for in EPA's upcoming health effects test guideline, *TSCA Acute Inhalation Toxicity with Histopathology*, which is the acute protocol to be required in the proposed HAPs Test Rule. EPA notes that the Alarie respiratory sensory irritation assay may be superfluous under an acceptable ECA, since additional PK and mechanistic data would be obtained.

**Subchronic toxicity testing:**

X<sup>b</sup> The MA Panel proposes to perform macrophage function tests and a 90-day study using three exposure concentrations and controls. A satellite group of animals will be used to evaluate recovery of highest exposure group.

X<sup>2</sup> Under an acceptable ECA, EPA can accept the proposed 90-day inhalation study with satellite group to explore recovery. EPA also strongly suggests additional interim sacrifices to provide data to inform about the choice of dose metric.

**Neurotoxicity testing (A & SC):**

-<sup>c</sup> No acute or subchronic neurotoxicity testing is proposed based on the premise that inhalation of MA will not result in toxicologically-significant blood levels of maleic acid. No oral or inhalation neurotoxicity data are available on which to base this comparison.

P(R)<sup>3</sup> EPA maintains that there are not sufficient data on either acute or subchronic inhalation neurotoxicity of MA and believes this proposed HAPs Test Rule testing requirement is needed. However, under an acceptable ECA, EPA could agree to reconsider the need for neurotoxicity testing if certain triggers are met. These triggers might provide that (1) blood levels of MA or maleic acid are not sufficient to warrant concern after inhalation exposures to MA in the PK studies, and (2) significant portal-of-entry effects are associated with these MA and maleic acid blood levels. EPA believes predictions using a PK model would also inform t&e agency about these considerations. EPA believes that, as an alternative, under an acceptable ECA, these studies could be performed via the oral route, if quantitative route-to-route extrapolation can be developed. See section 4 for additional details.

#### **Developmental toxicity testing :**

1 species Testing proposed in a mammalian species other than the rat.

- 4 No developmental toxicity testing is proposed based on the premise that inhalation of MA will not result in toxicologically-significant blood levels of maleic acid. Route-to-route comparison is proposed to be based on the blood levels of maleic acid associated with the NOAEL effect **level** for reproductive effects from this study (20 mg/kg).
- P(R)<sup>4</sup> EPA believes that there are not sufficient data on **the** developmental toxicity of inhalation exposures to MA that address this data need and believes the proposed **HAPs** Test Rule testing is needed. However, under an acceptable ECA, EPA could agree, to reconsider the need for developmental toxicity testing if certain triggers are met. These triggers might provide that (1) blood levels of MA or maleic acid are not sufficient to warrant concern **after** inhalation exposures to MA in the PK study, and (2) **significant** portal-of-entry effects are associated with these MA and maleic acid blood levels. If, based on **the** relevant information, the triggers are not met, EPA will maintain that developmental toxicity testing in a species other than the rat is needed **as** described in the proposed **HAPs** Test Rule. See Section 4 for additional details.

#### **Immunotoxicity screen:**

- 5 No SRBC is proposed. No immunotoxicity testing is **proposed** based on the premise that **inhalation** of MA will not result in toxicologically-significant blood levels of maleic acid. No oral or **inhalation** immunotoxicity data are available **on** which to base **this comparison**.
- 5 EPA believes that the SRBC assay is needed as described in the proposed HAPs Test Rule and suggests that a sensitization study be performed in guinea pigs to measure airway resistance and serum **globulins** to MA, MA-guinea pig serum albumin (GPSA) and GPSA before and **after** induction with either PA or hen egg **ovalbumin** as a **positive** control. See section 4 for additional details.

#### **Cancer/Genetox testing:**

- 1 Gas-phase exposure testing (Pegram et al., 1996) with *S. typhimurium* is proposed to avoid the problem of exposure to maleic acid rather than MA. The MA Panel proposal asserts that DNA acylation by MA is not expected to occur under physiological or any **other aqueous reaction conditions**.
- P<sup>7</sup> Under an acceptable ECA, the demonstration of the lack of mutagenicity and DNA **binding**, together with identification of a NOAEL for cytotoxicity of MA in the 90-day study, **as well as characterization of the (C x t) considerations of effect and recovery may be sufficient to allow EPA to reconsider the proposed HAPs Test Rule testing requirement for a two-year cancer bioassay**; If **sufficient** blood levels of MA or maleic acid to warrant concern for remote effects are demonstrated in the inhalation PK study, then EPA notes that the proposed PK work and model would provide predictions that could serve to inform the Agency about the comparison of measured and predicted blood **levels with effect levels** from the existing, oral, cancer bioassay (CIIT, 1984).
- EPA maintains that the proposed gas-phase testing in *S. Typhimurium* as well as DNA binding assays, should be conducted with a positive control, *i.e.* a known acylating agent, such as dimethylcarbamoyl chloride (DMCC). See section 4 for additional details.

#### **(4) EPA Comments on MA Panel Proposed PK Strategy**

EPA has reviewed the proposal for a **PK** strategy to address the data requirements on MA. This section provides detailed comments on the various components of the proposal and **summarizes** requirements that must be made in order for the proposal to be found acceptable.

In general, EPA agrees with the proposed mode of action and **dosimetry** considerations pertinent to evaluating the testing requirements for MA. EPA agrees that the **portal-of-entry** effects may be critical and delimiting, and maintains that the PK data be used to confirm that repeated inhalation exposures do not result in circulating MA or maleic acid levels to warrant concern (i.e., do not achieve MA or maleic acid levels associated with systemic endpoints).

**PK Model:** EPA agrees with the proposed mode of action and dose **metrics** for characterizing respiratory versus remote toxicity, i.e., maleic anhydride and maleic acid. EPA also agrees with the proposed **PK** model but disagrees with the limited nature of the duration and concentration range proposed for **the** effort. EPA believes that the MA Pa& must confirm that MA is so reactive that establishment of periodicity is not a consideration. For inhalation studies, confirmation should include repeated inhalation exposures' and blood analysis as a time course. EPA notes that urine concentrations may be needed to establish mass balance of maleic acid after periodic exposures. EPA also believes that more than one exposure concentration should be used, and that the **doses** selected parallel those selected for the proposed 90day inhalation study in order to facilitate comparison. between inhalation MA exposure levels that may be associated with **respiratory tract** toxicity and resultant MA or maleic acid blood levels that may be associated with systemic toxicity. In addition, EPA believes that these **same concentrations** should be used in the URT extraction study at more **than one** flow rate.

EPA notes that the MA Panel proposal does not address the mode of administration and vehicle **for the** oral PK **studies**. EPA notes **that this** should be **gavage** in corn oil to mimic that used for the oral studies of developmental and' reproductive toxicity (Jessup et al., 1982; Short et al., 1986) that are proposed **to** serve as the basis of route-to-route extrapolation. EPA agrees that the proposed approach will, probably provide sufficient data to determine whether or not the bloodlevels from inhalation exposures at remote sites are likely to result in toxicity relative to those exposures associated with portal-of-entry toxicity. This **judgement could** be made using triggers which might provide that (1) blood levels of MA or maleic acid are not sufficient to 'warrant concern **after** inhalation exposures to MA in the **PK** studies, and (2) significant port&of-entry effects are associated with these MA and maleic acid blood levels. If, based on the relevant information; the triggers are **not met**, then **PK** studies with a repeated exposure regimen and **additional** model development will be needed.

**Acute and Subchronic Toxicity Testing:** **While** EPA agrees that MA is established as **an irritant and sensitizer**, **the purpose** of the **HAPs** Test Rule is to acquire data that allows

characterization of the dose-response for various endpoints after inhalation exposure. EPA believes that the dose-response of acute inhalation MA exposures has not been adequately characterized. However, because no treatment-related systemic effects were demonstrated at exposure concentrations  $\leq$  21 ppm in a 4-week inhalation in rats (Goldenthal et al., 1984) or  $\leq$  2.5 ppm in the 3 species (rat, hamster, monkey) tested by Short et al. (1988) for 6 months, EPA agrees that the acute testing can 'be focused on characterizing the dose-response in the respiratory tract. In addition, EPA notes the liver and kidney must also be examined since **these are the** target tissues of MA or maleic acid. The acute inhalation study should include histopathology for the respiratory tract, liver, and kidney, and BAL assay as called for in EPA's upcoming health effects test guideline, **TSCA Acute Inhalation Toxicity with Histopathology**, which is the acute protocol to be required in the proposed HAPs Test Rule. EPA agrees with the MA Panel that **macrophage** function testing **is** needed as described in the previously mentioned health effects test guideline. EPA notes that the Alarie respiratory sensory irritation assay (ASTM E 981-84) may be superfluous under an acceptable ECA, since additional PK and mechanistic data would be obtained.

EPA agrees with the MA Panel's proposed **90-day** inhalation study with the satellite group to consider recovery as one means to allow the Agency to reconsider the proposed **HAPs** Test Rule testing need for carcinogenicity testing. EPA strongly suggests that additional interim sacrifices would provide insight on whether concentration (C), duration (t), or **the** (C x t) product is the dominant determinant of toxicity and, thereby provide information regarding the choice of appropriate dose metric.' The 90day inhalation study should include histopathology for the respiratory tract, liver, and kidney. The **90-day** inhalation study should also identify a NOAEL for **cytotoxicity**. EPA suggests that a satellite group to study recovery of lesions would enhance **evaluation** of the MA Panel's assertion-that carcinogenicity of MA in the respiratory tract is not likely. The development of this data may allow the Agency a means to reconsider the need for carcinogenicity testing as described in the proposed **HAPs** Test Rule. **If** the 90day study identifies **the** NOAEL for nasal irritation, and the absence of mutagenicity or DNA binding is demonstrated for MA, it could be argued that potential **tumors** would have to result from cytotoxicity and subsequent **cellular** proliferation as precursor events (see **carcinogenicity/genotoxicity** section);

**Neurotoxicity Testing:** EPA believes that there are not **sufficient** data on either acute or subchronic inhalation neurotoxicity of MA to address this data need and that this proposed HAPS Test Rule testing is needed. However, under an acceptable ECA, EPA could agree to reconsider the need for neurotoxicity testing if certain triggers are met. These triggers might provide that (1) blood levels of MA or maleic acid are not sufficient to warrant concern after inhalation exposures to MA **in** the PK studies, and (2) significant **portal-of-entry** effects are **associated** with these MA and maleic acid blood levels. EPA believes predictions using a PK model would also inform the Agency about these considerations. The significance of MA or maleic acid levels in the blood after **inhalation** exposure in the PK studies will be judged in comparison to the blood levels obtained with oral dosing in the **PK** studies and in comparison

to effect levels in acute and subchronic studies (existing studies as well as ECA studies). EPA has no knowledge of neurotoxicity testing data available on MA or maleic acid after oral exposures that could be used for comparison with MA or maleic acid blood levels achieved after inhalation of MA. If, based on the relevant information, the triggers are not met, then EPA will maintain that the acute and subchronic inhalation neurotoxicology battery is needed as described in the proposed **HAPs** Test Rule. As an alternative, under an acceptable ECA, these studies could be performed via the oral route, if quantitative route-to-route extrapolation can be developed.

**Developmental Toxicity Testing:** EPA maintains that there are not sufficient data on the developmental toxicity of inhalation exposures to MA and believes that the developmental toxicity testing as described in the proposed **HAPs** Test Rule 'is needed. However, under an acceptable ECA, EPA could agree to reconsider the need for developmental toxicity testing if certain triggers are met. These triggers might provide that (1) blood levels of MA or maleic acid are not sufficient to warrant concern after inhalation exposures to MA in the PK studies, and (2) significant **portal-of-entry** effects are associated with these MA and maleic acid blood levels. EPA believes that predictions using a PK model would also inform the Agency about these considerations. The significance of MA or maleic acid levels in the blood after inhalation exposure in the PK studies will be judged in comparison to the blood levels obtained with oral dosing in the PK studies and in comparison to effect levels in the acute and subchronic studies (existing studies as well as ECA studies). If, based on the relevant information, the triggers are not met, then EPA will maintain that developmental toxicity testing in a species other than the rat is needed as described in the proposed **HAPs** Test Rule. EPA believes that the proposed route-to-route comparison of the effect levels in the developmental study in rats by Short et al. (1986) would inform the Agency about consideration of the likelihood **for developmental** effects after inhalation exposures in rats, and that additional PK studies using repeated exposure regimen and additional model development may be needed. EPA notes that a rigorous review and designation of the effect levels in Short et al. (1986) is required since on preliminary analysis a slight trend with dose is noted in fetal weight, **unossified** sternebrae, and pregnancy rate. This preliminary EPA analysis suggests that effects may have occurred in this study. EPA notes that the MA **Panel's** proposal does not address how the second species testing need for developmental toxicity testing (other than the rat), will be met as identified in the proposed **HAPs** Test Rule.

**Immunotoxicity Screen:** EPA is not convinced that the rat is an appropriate model for MA **sensitization** and notes that the guinea pig is established as the test species for this endpoint. EPA's concern is born out by the weakly positive (minimal LOAEL) results observed in the cited CIIT Research Institute (1991) investigation in rats in the face of human data showing **that** MA is a sensitizer and is capable of causing cross-reactivity with other anhydrides (Baur et al., 1995). Part of the insensitivity of this model may also be due to the fact that only **IgG** and not **IgE** antibody levels were determined in the rat. EPA maintains that, although established as a potent sensitizer, the dose-response of MA for this effect has not been well characterized. EPA suggests that a sensitization study as proposed for phthalic anhydride

(CMA, 1996) be performed in guinea pigs to adequately address this concern. The study proposed therein measures airway resistance and serum globulins to PA, PA-guinea pig serum albumin (GPSA) and GPSA before and after induction with either PA or hen egg ovalbumin as positive control.

Further, due to MA's demonstrated immunotoxic activity, EPA maintains that the SRBC assay is needed as described in the proposed HAP's Test Rule in order to characterize potential effects on other aspects of **immune** function. EPA believes that circulating cytokines or antibodies secondary to the demonstrated portal-of-entry effects could have systemic effects.

**Carcinogenicity/Genetox Testing:** EPA remains concerned about the possibility that MA may be carcinogenic via the inhalation route. Both dimethylcarbamoyl chloride (DMCC) and diethyl carbamoyl chloride (DECC), two direct-acting acylating rodent carcinogens, have been demonstrated to form DNA **adducts** in vitro at **pH 7.0-7.5** and **37 °C** (Segal et al. 1982). Both **bis(chloromethyl)ether** (a direct-acting alkylating agent) and DMCC are hydrolyzed rapidly under aqueous conditions, yet both are potent inhalation carcinogens. MA is not expected to be as potent a carcinogen as DMCC, but it does have the potential to bind to DNA under, physiological conditions so that the potential cancer hazard by the inhalation route must be characterized.

EPA agrees with the merit of the proposed gas-phase **testing** in ***S. Typhimurium***. However, because the efficacy of this system for testing acylating agents is unknown, EPA maintains that the use of a positive control with a known acylating agent, such as dimethylcarbamoyl chloride (**DMCC**) **should** be incorporated into this test protocol. In addition, DNA binding assays, again with an acylating agent, such as DMCC as a positive control, should be performed to rule out the concern for acylation.

Under an acceptable ECA, the demonstration of the lack of mutagenicity and DNA binding, together with **identification** of a NOAEL for **cytotoxicity** of MA in the **90-day** inhalation study, as well as **characterization** of the (C x t) considerations of effect and recovery may be sufficient to allow EPA to reconsider the requirement for a **two-year** cancer bioassay.

If sufficient blood levels of MA or **maleic** acid to warrant concern for remote effects are demonstrated in the inhalation **PK** study, then EPA notes that the proposed **PK** work and model would provide predictions that could serve to inform the Agency about the comparison of measured and predicted blood levels **with** effect levels from the existing **oral** cancer bioassay (**CIIT**, 1984)

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**(6) PK Proposal Review Staff**

The following table lists individuals who contributed in the preparation of EPA's preliminary technical analysis of the Chemical Manufacturers Association's **Maleic** Anhydride Panel PK proposal for **maleic** anhydride.

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